

Effect of Processing Conditions on Phytic Acid, Calcium, Iron, and Zinc Contents of Lime-Cooked Maize

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Tortillas are made by cooking maize in a lime solution during variable times and temperatures, steeping the grain for up to 12 h, washing and grinding it to a fine dough, and cooking portions as flat cakes for up to 6 min. The effects of the main processing steps on the chemical composition, nutritive value, and functional and physicochemical characteristics have been areas of research. The present work evaluates the effect of lime concentration (0, 1.2, 2.4, and 3.6%) and cooking times (45, 60, and 75 min) on phytic acid retention of whole maize, its endosperm, and germ, as well as on the content of calcium, iron, and zinc on the same samples. The effects of steeping time and temperature and steeping medium on the phytic acid of lime-cooked maize were also studied. Finally, phytic acid changes from raw maize to tortilla were also measured. The results indicated that lime concentration and cooking time reduce phytic acid content in whole grain (17.4%), in endosperm (45.8%), and in germ (17.0%). Statistical analyses suggested higher phytic acid loss with 1.2% lime and 75 min of cooking. Cooking with the lime solution is more effective in reducing phytic acid than cooking with water. Steeping maize in lime solution at 50 °C during 8 h reduced phytic acid an additional 8%. The total loss of phytic acid from maize to tortilla was 22%. Calcium content increased in whole maize, endosperm, and germ with lime concentration and cooking and steeping times. The increase was higher in the germ than in the endosperm. The level, however, can be controlled if steeping of the cooked grain is conducted in water. Iron and zinc contents were not affected by nixtamalization processing variables but were affected in steeping.

KEYWORDS: Maize; lime cooking; processing variables; phytic acid; calcium; iron; zinc; whole grain; endosperm; germ

INTRODUCTION

Maize is the main cereal grain consumed by large sectors of the rural and urban populations in Central America and Mexico (1). Its intake is relatively high, providing young and mature individuals significant amounts of calories, protein, and other nutrients (2). Maize is converted into edible products in these countries through the nixtamalization process, which has been reviewed by various researchers from technological, physical, chemical, and nutritional points of view (3, 5). The main food product derived is the maize tortilla, which is produced by cooking maize in lime water during ~50–75 min, followed by steeping during 10–12 h. The cooked grain is washed to remove excess lime and solids from the grain and then converted into a dough that is then used to bake the tortillas. This process is still carried out in rural and urban households; however, in recent years industrial nixtamalized maize flour has become available and is being increasingly used for the preparation of tortilla and other nixtamalized maize products (1).

Due to the importance of nixtamalized maize flour for food and nutrition, its commercial availability, and consumer acceptance, efforts are being made to fortify it with micronutrients, particularly those deficient in rural diets. A number of studies have been published on the loss of vitamins due to the alkaline cooking process (5) as well as on the changes in mineral concentrations (3, 5) and calcium and niacin bioavailability (3, 5). Likewise, some limited efforts were done in the past to fortify nixtamalized maize flour with protein and micronutrients (1, 6). Fortification attempts for nixtamalization of maize flour present some important challenges, not so much with the vitamins but mainly with minerals, particularly iron, due to the presence of organic compounds in maize such as phytic acid, which exert inhibitory actions on its bioavailability (7–9).

Data on phytic acid content in nixtamalized maize flour are available, with values varying from 0.5 to 0.9% (7–12). However, specific studies to establish the effect of processing variables during nixtamalization on phytic acid are very few. In one study, Urizar and Bressani (12) showed phytic acid to decrease some 35% due to both calcium hydroxide concentration added during cooking and cooking time. In a second study with 11 maize varieties, Bressani et al. (4) found losses of phytic

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acid up to 28%. Gomez-Aldapa et al. (11) reported losses of phytic acid when maize was extruded for tortilla flour. The phytic acid losses were 29.9% for lime-cooked flour; 31% when extrusion was conducted without the addition of lime, 36.1% with 0.15% lime addition, and 31.9% with 0.25% lime addition on extrusion. Therefore, this process did not decrease phytic acid much more than traditional nixtamalization. Khan et al. (13) reported a 23.7% loss of phytic acid in maize processed by roasting and a loss of only 18.9% by boiling. The present study evaluates the effects of lime level and cooking time on the contents of phytic acid, calcium, zinc, and iron of the whole cooked grain as well as in the germ and endosperm fractions. The effect of cooking with and without lime and the importance of temperature and time of soaking on the phytic acid content of the whole cooked grain were also studied, as well as possible changes in phytic acid content during the transformation of cooked grain into a dough achieved by grinding and cooking the dough into a tortilla through a short cooking process.

MATERIALS AND METHODS

The study was conducted with a semihard white corn hybrid HB-83 commonly used by farmers of the tropical lowlands of Guatemala for making tortillas. The sample was directly purchased from a farmer, brought to the laboratory, and kept at 6 °C until used. This hybrid has a density of 1.29 ± 0.01 g/mL, with a 1000 grain weight of 307.53 ± 4.50 g, $4.33 \pm 0.58\%$ floaters, and a moisture content of $13.11 \pm 0.12\%$. The pericarp represented $5.51 \pm 0.12\%$, the germ $9.63 \pm 0.10\%$, and the endosperm $84.85 \pm 0.58\%$ of the kernel weight.

Because the nixtamalization process involves two main operations, the effect of the processing variables on phytic acid retention was studied by stages. The first operation involves cooking the grain with lime, whereas the second operation is that of steeping the cooked maize without any additional heat.

For the grain-cooking operation the two variables studied were lime concentration (0, 1.2, 2.4, and 3.6% of lime of maize weight) and cooking time (45, 60, and 75 min) for each level of lime, with two replications per treatment. Cooking of 200 g of maize to which 600 mL of water was added was conducted at 94 °C. At the end of cooking, the maize was washed with water two or three times to remove excess lime and the free pericarp of the cooked kernel. The 200 g cooked sample was divided at the end of cooking into two portions of 133 and 67 g each. The large subsample was used to obtain the germ and the endosperm so that from each processing treatment there were three samples: the whole cooked grain and the cooked germ and endosperm. The germ was manually separated from the endosperm by inserting a sharpened spatula between the germ and endosperm. All subsamples were dried with air at 65 °C to constant weight and ground when dried. They were then stored at 6 °C.

The second study consisted of evaluating the effect of steeping variables on the phytic acid, calcium, iron, and zinc contents of the cooked grain. The maize variety HB-83 was cooked with 1.2% lime based on maize weight, in 3 volumes of water during 75 min at 94 °C. After cooking, the maize samples were soaked in their own cooking liquor or in water for 0, 2, 5, and 8 h at two temperatures: 25 and 50 °C. After the applications of the treatments indicated, the samples were dried with air at 65 °C and then ground.

The last study dealt with processing maize as previously indicated (1.2% lime, 75 min, 94 °C, 8 h of soaking in the alkaline solution). Samples were obtained at the beginning, after lime water cooking, after steeping, after grinding into a dough, and as the tortilla and were dried with air at 65 °C and analyzed for phytic acid.

Raw and processed samples of each experiment dried as indicated above were analyzed in duplicate, for moisture by AOAC method 10.138 (13), for Ca, Fe, and Zn by atomic absorption, AOAC method 968.08 (14), and for phytic acid according to the method of Hough and Lantzsch (15). All data are expressed on an oven-dry weight basis.

The statistical program SAS version 8 (MS Windows) was used to analyze the results.

Table 1. Phytic Acid Changes in the Whole Grain, Endosperm, and Germ with Respect to Lime Concentration and Cooking Time during Nixtamalization (mg %)^a

lime level (%)	cooking time				av
	raw	45 min	60 min	75 min	
Whole Grain					
0	733 ± 6	691 ± 56	688 ± 11	682 ± 5	699 a
1.2	733 ± 6	634 ± 8	630 ± 21	604 ± 40	650 b
2.4	733 ± 6	624 ± 8	619 ± 42	609 ± 28	646 b
3.6	733 ± 6	620 ± 46	608 ± 48	608 ± 32	642 b
av	733 a	642 b	636 b	626 b	
Endosperm					
0	181 ± 19	172 ± 26	156 ± 30	156 ± 18	167 a
1.2	181 ± 19	121 ± 23	118 ± 12	101 ± 22	130 b
2.4	181 ± 19	110 ± 23	109 ± 22	99 ± 24	125 b
3.6	181 ± 19	103 ± 0	105 ± 30	98 ± 18	122 b
av	181 a	127 b	122 b	113 b	
Germ					
0	4283 ± 34	4195 ± 364	4190 ± 112	4160 ± 344	4207 a
1.2	4283 ± 34	3721 ± 333	3697 ± 142	3581 ± 765	3820 ab
2.4	4283 ± 34	3935 ± 94	3677 ± 403	3556 ± 211	3862 ab
3.6	4283 ± 34	3687 ± 225	3282 ± 316	3562 ± 180	3709 b
av	4283 a	3891 ab	3711 b	3714 b	

^a Oven-dried weight basis.

RESULTS AND DISCUSSION

Effect of Lime Concentration and Cooking Time. The results of this study on phytic acid content in the whole grain as well as in the germ and endosperm are shown in **Table 1**. The level of lime that resulted in the highest loss of phytic acid was 1.2% (14.8%), whereas only a small increase resulted from the use of 3.6% lime (16.3% phytic acid). On the other hand, cooking time during 75 min resulted in a 14.9% loss of phytic acid in the whole grain. The largest loss of phytic acid was observed when using 1.2% lime and a 75 min cooking time. The effects of lime and cooking time were statistically significant ($p < 0.005$). The loss in phytic acid in this study was lower than that previously reported by Urizar and Bressani (12).

The phytic acid change in the endosperm when the whole grain was cooked with up to 3.6% lime and for 75 min is also shown in **Table 1**. Cooking time reduced phytic acid from 181 mg % in raw endosperm to lower values at 75 min of cooking depending on the level of lime used. Lime concentration of 1.2% with 75 min of cooking gave the highest losses in the whole grain and induced a loss of 44.2% in endosperm. Lime concentration gave statistically significant effects ($p < 0.005$) as well as cooking time. The phytic acid values for the germ are also shown in **Table 1**. This morphological fraction has been shown to contain >90% of the maize grain phytic acid (18, 19), as is also the case in this study. Lime level accounted for a loss of 18.1%, whereas cooking time induced a loss of 13.3%. A loss of 16.4% was observed when lime concentration was 1.2% with 75 min of cooking time. This loss was higher when lime concentration was 3.6% for a 60 min cooking time. Statistical analysis indicated lime level and cooking time to induce significant effects on phytic acid content. Lime content at a fixed cooking time of 75 min decreases phytic acid by ~20% of the original value. On the other hand, with 1.2% lime, a cooking time of up to 75 min reduced phytic acid some 25% of the initial value.

Table 2 summarizes the calcium content in whole maize, endosperm, and germ when the grain was cooked with increasing lime levels and with increasing cooking time. With the whole kernel, lime concentration increased calcium content from 9.0

Table 2. Calcium Content Changes in the Whole Grain, Endosperm, and Germ with Respect to Lime Concentration and Cooking Time during Nixtamalization (mg %)^a

lime level (%)	cooking time				
	raw	45 min	60 min	75 min	av
Whole Grain					
0	8.4 ± 0.07	8.4 ± 0.4	8.1 ± 2.3	11.2 ± 1.8	9.0 b
1.2	8.4 ± 0.07	143.4 ± 8.9	187.0 ± 17.8	205.4 ± 35.4	136.2 a
2.4	8.4 ± 0.07	162.3 ± 0	177.8 ± 47	172.5 ± 29.4	130.2 a
3.6	8.4 ± 0.07	171.3 ± 4.1	212.0 ± 26.4	240.2 ± 22.1	158.6 a
av	8.4 a	121.9 b	146.2 b	157.4 b	
Endosperm					
0	5.2 ± 0.35	5.0 ± 0.2	5.5 ± 0.6	6.2 ± 1.1	5.5 b
1.2	5.2 ± 0.35	43.0 ± 0.8	55.8 ± 2.1	63.5 ± 1.8	41.9 a
2.4	5.2 ± 0.35	52.4 ± 0	66.2 ± 8.8	68.5 ± 1.8	48.1 a
3.6	5.2 ± 0.35	50.4 ± 2.8	69.8 ± 10.6	79.8 ± 3.7	51.3 a
av	5.2 c	37.7 b	49.3 ab	54.5 a	
Germ					
0	15.0 ± 1.06	14.3 ± 1.8	15.5 ± 0	14.3 ± 4.5	14.8 b
1.2	15.0 ± 1.06	296.5 ± 121.1	355.1 ± 17.1	272.7 ± 55.4	23.4 a
2.4	15.0 ± 1.06	369.9 ± 50.3	295.1 ± 99.7	304.1 ± 99.8	246.2 a
3.6	15.0 ± 1.06	268.7 ± 38.3	257.1 ± 55.8	254.2 ± 0	198.9 ab
av	15.0 b	237.4 a	230.9 a	211.5 a	

^a Oven-dried weight basis.

to 158.6 mg/100 g, but the largest increase took place with 3.6% lime. Cooking time also increased calcium content at 45 min and only slightly more with 60 and 75 min of cooking. The effects of lime concentration and cooking time were statistically significant. The results with the endosperm also showed an increase with cooking time and lime concentration, and both gave highly statistically significant ($p < 0.005$) effects.

With germ, a statistically significant increase in calcium content was observed with 1.2 and 2.4% lime, which was proportionately slightly higher than in the whole grain but significantly higher in absolute amounts. Higher levels of calcium were retained in the germ at 45 and 60 min than at 75 min. Calcium absorption in the germ was significantly higher than that observed in the endosperm. Similar results have been previously informed (3, 4), but no explanation has been given for this finding. Losses of ether extract have been reported for lime cooking of maize, which may be due to reactions of free fatty acid with calcium (3). It is also of interest to speculate on the germ being a high source of phytic acid (18, 19) and a tissue that absorbs high levels of calcium (3, 4). Trejo-Gonzalez et al. (16) indicate that the calcium taken up by the corn grain during cooking appeared to be bound to the starch grain, because the starch isolated from the lime-treated maize took up ~3 times more Ca than starch isolated from untreated maize. Serna-Saldivar et al. (3) showed the germ to retain more calcium than the endosperm. Recently, Zazueta et al. (17), using radiolabeled calcium ions, showed that Ca was taken up rapidly by the grain and was fixed in the outer boundary of the endosperm, especially at the external surface of the germ. After extended steeping times, a moderate amount of ⁴⁵Ca was evident in the germ as shown in the present study.

The effects of lime concentration and cooking time on iron and zinc contents with whole grain and in endosperm and germ are presented in **Tables 3** and **4**. The results for both mineral elements are similar in that neither lime concentration for cooking nor cooking time affected the levels in the cooked whole grain or in the germ. The effects were statistically not significant. However, cooking time influenced significantly iron content in the endosperm.

Table 3. Iron Content Changes in the Whole Grain, Endosperm, and Germ with Respect to Lime Concentration and Cooking Time during Nixtamalization (mg %)

lime level (%)	cooking time				
	raw	45 min	60 min	75 min	av
Whole Grain					
0	2.65 ± 0.23	2.11 ± 0.35	1.93 ± 0.45	1.87 ± 0	2.14 a
1.2	2.65 ± 0.23	2.30 ± 0.26	2.30 ± 0.26	2.18 ± 0.26	2.36 a
2.4	2.65 ± 0.23	2.12 ± 0.18	1.87 ± 0.17	2.05 ± 0.26	2.17 a
3.6	2.65 ± 0.23	2.24 ± 0.71	2.30 ± 0.45	2.30 ± 0.26	2.37 a
av	2.65 a	2.19 a	2.10 a	2.10 a	
Endosperm					
0	1.94 ± 0.23	1.18 ± 0.44	1.31 ± 0.08	0.81 ± 0.27	1.31 a
1.2	1.94 ± 0.23	1.06 ± 0.08	1.18 ± 0.08	0.81 ± 0.08	1.24 a
2.4	1.94 ± 0.23	1.24 ± 0.18	1.62 ± 0.35	0.87 ± 0.18	1.42 a
3.6	1.94 ± 0.23	1.61 ± 0.70	1.30 ± 0.26	0.99 ± 0.18	1.46 a
av	1.94 a	1.27 ab	1.35 ab	0.87 b	
Germ					
0	10.33 ± 0.52	9.97 ± 0.88	8.71 ± 0.88	8.74 ± 0	9.44 a
1.2	10.33 ± 0.52	9.68 ± 0.43	8.74 ± 0.01	9.67 ± 0.43	9.60 a
2.4	10.33 ± 0.52	9.35 ± 0.02	9.04 ± 0.44	9.67 ± 0.46	9.60 a
3.6	10.33 ± 0.52	9.35 ± 0.01	9.98 ± 0.91	9.97 ± 0.86	9.91 a
av	10.33 a	9.58 a	9.11 a	9.51 a	

Table 4. Zinc Content Changes in the Whole Grain, Endosperm, and Germ with Respect to Lime Concentration and Cooking Time during Nixtamalization (mg %)

lime level (%)	cooking time				
	raw	45 min	60 min	75 min	av
Whole Grain					
0	2.24 ± 0.05	2.49 ± 0.35	2.50 ± 0.35	2.24 ± 0	2.36 a
1.2	2.24 ± 0.05	2.62 ± 0.52	2.49 ± 0.36	2.37 ± 0.18	2.43 a
2.4	2.24 ± 0.05	2.50 ± 0.35	2.24 ± 0.01	2.22 ± 0.31	2.30 a
3.6	2.24 ± 0.05	2.49 ± 0.71	2.49 ± 0.36	2.34 ± 0.13	2.39 a
av	2.24 a	2.52 a	2.43 a	2.29 a	
Endosperm					
0	0.88 ± 0.18	0.99 ± 0.53	0.75 ± 0	0.68 ± 0.10	0.82 a
1.2	0.88 ± 0.18	0.87 ± 0.18	0.87 ± 0	0.75 ± 0.08	0.84 a
2.4	0.88 ± 0.18	0.87 ± 0.18	0.87 ± 0	0.68 ± 0.10	0.83 a
3.6	0.88 ± 0.18	0.94 ± 0.26	0.75 ± 0	0.75 ± 0	0.83 a
av	0.88 a	0.92 a	0.81 a	0.72 a	
Germ					
0	11.79 ± 0.95	12.46 ± 0	12.44 ± 0	12.49 ± 0.01	12.29 a
1.2	11.79 ± 0.95	11.21 ± 1.10	11.71 ± 1.12	11.69 ± 1.09	11.72 a
2.4	11.79 ± 0.95	12.46 ± 0.03	12.47 ± 0.01	12.47 ± 0.03	12.29 a
3.6	11.79 ± 0.95	12.41 ± 0.01	12.47 ± 0.04	13.25 ± 1.08	12.49 a
av	11.79 a	12.27 a	12.27 a	12.47 a	

Effect of Steeping after Cooking. As indicated above, the transformation of maize into nixtamalized flour or tortilla consists of two processing steps, at least for the traditional Mayan technology. One of the steps, which has been already reported, is the cooking process with lime, and the second step is the steeping process. The soaking effects were then studied and, for these, lime concentration for cooking in water was set at 1.2% in one case and no lime in another, with a cooking time of 75 min at a cooking temperature of 94–96 °C. After cooking, the maize weight from each cooking operation was divided into four portions. One was allowed to steep in its own cooking liquor (alkaline soaking) for 0, 2, 5, and 8 h at 25 °C, whereas a second was allowed to soak at 50 °C during the same periods of time. The other two portions were soaked during the same periods of time and temperature but in distilled water rather than in lime water.

Table 5. Phytic Acid Content in Alkaline Solution and in Water-Cooked Maize Subjected to Different Steeping Conditions (mg %)

cooking variable	steeping medium	<i>T</i> (°C)	raw maize	cooked maize steeping time			
				0 h	2 h	5 h	8 h
lime solution	alkaline	25	765 ± 15	598 ± 24	566 ± 13	554 ± 17	551 ± 27
		50	765 ± 15	598 ± 24	555 ± 24	553 ± 11	546 ± 7
	water	25	765 ± 15	598 ± 24	555 ± 31	551 ± 13	545 ± 23
		50	765 ± 15	598 ± 24	550 ± 7	544 ± 38	540 ± 18
water	alkaline	25	765 ± 15	662 ± 26	659 ± 27	660 ± 19	662 ± 14
		50	765 ± 15	662 ± 26	662 ± 26	645 ± 34	653 ± 30
	water	25	765 ± 15	662 ± 26	669 ± 3	660 ± 13	655 ± 19
		50	765 ± 15	662 ± 26	654 ± 0	654 ± 3	654 ± 19

Table 6. Calcium Content in Maize Cooked either in Lime Solution or in Water and Subjected to Different Steeping Conditions (mg %)

cooking variable	steeping medium	<i>T</i> (°C)	raw maize	cooked maize steeping time			
				0 h	2 h	5 h	8 h
lime solution	alkaline	25	8.9 ± 1.69	76.7 ± 9.55	109.7 ± 15.2	149.8 ± 6.2	194.6 ± 19.5
		50	8.9 ± 1.69	76.7 ± 9.55	123.6 ± 3.3	156.9 ± 14.6	204.9 ± 22.9
	water	25	8.9 ± 1.69	76.7 ± 9.55	85.5 ± 0	82.6 ± 4.0	80.6 ± 0
		50	8.9 ± 1.69	76.7 ± 9.55	83.1 ± 1.6	83.9 ± 0	64.9 ± 0
water	alkaline	25	8.9 ± 1.69	8.7 ± 1.91	9.5 ± 0.42	10.2 ± 0.7	9.3 ± 0.2
		50	8.9 ± 1.69	8.7 ± 1.91	8.9 ± 0.85	8.5 ± 0.4	10.0 ± 0.8
	water	25	8.9 ± 1.69	8.7 ± 1.91	9.1 ± 0.14	6.1 ± 0.8	10.0 ± 0
		50	8.9 ± 1.69	8.7 ± 1.91	9.4 ± 1.41	7.8 ± 0.9	11.6 ± 1.2

The dried samples were then analyzed for phytic acid, calcium, iron, and zinc. The phytic acid values are shown in **Table 5**. Raw maize had an average phytic acid content of 765 mg/100 g. At the end of cooking in lime equivalent to 0 h of steeping, the level of phytic acid was 598 ± 24 mg/100 g, representing a 21.8% loss, whereas at the end of cooking in water alone, the level of phytic acid was 662 ± 26 mg/100 g or a loss of 13.5%. Khan et al. (13) found a loss of 18.9% upon water cooking.

Steeping of the alkaline cooked maize for up to 8 h in an alkaline medium or in a water medium decreased phytic acid from 8.4 to 9.2%, for total phytic acid losses of 28.4% for alkaline soaking and 29.0% for water steeping at 8 h. Steeping temperature did not have an important influence in reducing the phytic acid level.

The phytic acid changes upon steeping of the water-cooked maize did not show an additional change from that which took place upon cooking.

Analysis of variance of the data in **Table 5** showed a highly significant effect ($p < 0.0001$) due to cooking in lime as well as a significant effect of steeping time for the lime-cooked maize. Two-, three-, and four-way interactions were not statistically significant. **Table 9**

shows the Tukey range test for these results.

The behavior of calcium is described in **Table 6**. Alkaline cooking induced an average increase in calcium to a level of 76.7 mg % from an initial value of 8.98 mg/100 g in raw maize. Soaking in the lime cooking solution during 8 h increased calcium content up to an average value of 199.6 mg % for both temperatures. The effects of soaking temperatures were for all practical purposes very similar but somewhat higher at 50 °C. Soaking the lime-cooked grain in water resulted in lower calcium values at both temperatures and during all soaking times when compared to the maize soaked in lime water. Analyses of variance showed a highly significant effect in increasing Ca

content due to cooking ($p < 0.0001$) and to soaking ($p < 0.0001$) as well as to soaking time ($p < 0.0001$) but not to soaking temperature. Two-way interactions were highly significant ($p < 0.0001$) for cooking × soaking, cooking × time, and soaking × time. Three-way interactions were highly significant ($p < 0.0001$) for cooking × soaking × time. There were no four-way interactions. Water cooking and soaking did not affect calcium level. **Table 9** shows the Tukey range test for these results.

Iron and zinc contents are shown in **Tables 7** and **8**. With iron, cooking in lime solution or in water and soaking in lime solution or in water at two soaking temperatures did not affect the levels found. However, steeping time induced a significant reduction (**Table 9**). For zinc there was a statistical difference due to cooking medium, but not for steeping medium or steeping temperature. However, steeping time induced statistically significant effects on Zn content (**Table 9**).

Changes in Phytic Acid from Raw Maize to Tortilla. For this final study maize was cooked with 1.2% lime for 75 min followed by 8 h of soaking in its own cooking liquor at room temperature. The samples were then washed with water and air-dried at 65 °C. The changes in phytic acid are shown in **Table 10**.

Lime cooking reduced phytic acid some 18.2%, which is similar to values presented earlier in this paper. The next sample was the cooked, soaked, and washed maize grain (nixtamal) with a phytic acid value of 596 ± 12.4 mg/100 g, which represents a loss of 19.9% of the original phytic acid content. This material is then ground into a dough, which gave a value of 591 ± 9.2 mg, giving a loss of phytic acid slightly higher than that of the nixtamal. Finally, the dough is baked into a tortilla with a phytic acid value of 585 ± 11.2 mg/100 g, equivalent to a 21.4% loss. This value is similar to values reported by Reinhold and Garcia (7, 8), Martinez-Torres et al. (9), and Wyatt and Triana-Trejos (10) but lower than the value

Table 7. Iron Content in Maize Cooked either in Lime Solution or in Water and Subjected to Different Steeping Conditions (mg %)

cooking variable	steeping medium	T (°C)	raw maize	cooked maize steeping time			
				0 h	2 h	5 h	8 h
lime solution	alkaline	25	2.18 ± 0.08	1.79 ± 0.09	1.60 ± 0.16	1.62 ± 0.06	2.07 ± 0.36
		50	2.21 ± 0.13	1.79 ± 0.09	1.85 ± 0	1.97 ± 0.02	1.55 ± 0.09
	water	25	2.18 ± 0.08	1.79 ± 0	1.78 ± 0.07	1.66 ± 0.07	1.86 ± 0
		50	2.21 ± 0.13	1.79 ± 0	1.92 ± 0.08	1.77 ± 0.25	1.71 ± 0.37
water	alkaline	25	2.18 ± 0.08	1.61 ± 0.007	1.73 ± 0.007	1.78 ± 0.06	1.80 ± 0.09
		50	2.21 ± 0.13	1.61 ± 0	1.77 ± 0.08	1.84 ± 0.16	2.04 ± 0.44
	water	25	2.18 ± 0.08	1.61 ± 0	2.01 ± 0.09	1.83 ± 0.01	1.83 ± 0
		50	2.21 ± 0.13	1.61 ± 0	2.41 ± 0.60	1.92 ± 0.53	1.82 ± 0.13

Table 8. Zinc Content in Maize Cooked Either in Lime Solution or in Water and Subjected to Different Steeping Conditions (mg %)

cooking variable	steeping medium	T (°C)	raw maize	cooked maize steeping time			
				0 h	2 h	5 h	8 h
lime solution	alkaline	25	2.10 ± 0.17	2.22 ± 0.007	1.98 ± 0.007	2.00 ± 0.42	2.31 ± 0.19
		50	2.10 ± 0.17	2.22 ± 0.007	2.23 ± 0.007	2.09 ± 0.19	1.74 ± 0.007
	water	25	2.10 ± 0.17	2.22 ± 0.007	1.72 ± 0.007	1.72 ± 0.007	1.98 ± 0
		50	2.10 ± 0.17	2.22 ± 0.007	2.11 ± 0.17	1.83 ± 0.17	1.71 ± 0.37
water	alkaline	25	2.10 ± 0.17	1.98 ± 0.007	2.10 ± 0.16	1.96 ± 0.32	1.86 ± 0.17
		50	2.10 ± 0.17	1.98 ± 0.007	1.84 ± 0.17	1.84 ± 0.16	1.86 ± 0.17
	water	25	2.10 ± 0.17	1.98 ± 0.007	1.94 ± 0	1.84 ± 0.16	1.95 ± 0
		50	2.10 ± 0.17	1.98 ± 0.007	2.11 ± 0.17	1.70 ± 0.32	1.84 ± 0.16

Table 9. Tukey's Range Test for the Results of the Cooking and Steeping Study

	phytic acid	calcium	iron	zinc
cooking medium				
in lime solution	680 a ^a	88 a	1.89 a	2.03 a
in water	603 b	9 b	1.86 a	1.93 b
steeping				
alkaline	643 a	60 a	1.90 a	2.00 a
water	641 a	37 b	1.85 a	1.96 a
T (°C) of steeping				
25	644 a	49 a	1.90 a	2.01 a
35	640 a	48 a	1.85 a	1.96 a
steeping time (h)				
raw	765 a	8.9 e	2.19 a	2.10 a
0	630 b	43 d	1.70 b	2.07 ab
2	609 bc	55 c	1.88 b	1.97 abc
5	602 c	63 b	1.76 b	1.87 c
8	601 c	73 a	1.83 b	1.90 bc

^a Different letters indicate statistically significant differences.

Table 10. Change in Phytic Acid Content from Raw Maize to Tortilla

sample	phytic acid (mg/100 g)	% change
raw	744 ± 20.9 a ^a	0
cooked maize	609 ± 9.2 b	18.2
nixtamal	596 ± 12.4 b	19.9
dough	591 ± 9.2 b	20.6
tortilla	585 ± 11.2 b	21.4

^a Different letters indicate statistically significant differences.

previously reported by Urizar and Bressani (12), although the percentage loss was very similar. Mendoza et al. (22) reported a loss of 14.3% phytic acid upon nixtamalization from a common maize sample, but there was no loss from nixtamalization for a maize mutant with 65% of the phytic acid content of the common maize.

The level of phytic acid found in this study after nixtamalization is sufficiently high to affect iron bioavailability. Martinez-Torres et al. (9) reported an iron absorption of $2.8 \pm 1.2\%$ when the phytate content was 565 g/100 g. Absorption increased to 4.0 ± 1.3 with 318 mg/100 g and to $5.8 \pm 1.2\%$ when the phytic acid content was 120 mg/100 g. The low phytic acid maize mutant used by Mendoza et al. (20) had a higher iron absorption than the normal maize. In the case of the lime-cooked tortilla the utilization of iron may be even lower because of the presence of relatively large amounts of calcium, known to reduce iron bioavailability (21, 22). However, soaking the cooked grain in water reduces the calcium content some 50–60% in commonly processed nixtamal (6). This procedure, however, may not be advisable because large population groups in Mexico and Central America depend on tortillas for their calcium intake. It would be of interest to study the bioavailability of iron from maize germ before and after nixtamalization.

LITERATURE CITED

- Bressani, R. Nutritional quality of nixtamalized corn more flour. Achievement through fortification with micronutrients. In *Fortification of Corn Masa Flour with Iron and/or Other Nutrients*; a literature and industry experience review; Sustain: Washington, DC, 1997.
- Encuesta Nacional de Consumo Aparente de Alimentos. Instituto Nacional de Estadística Secretaria General de Planificación Económica, Guatemala, 1991.
- Serna-Saldivar, S. O.; Gomez, M. H.; Rooney, L. W. The chemistry, technology and nutritional value of alkaline cooked corn products. Chapter 4. In *Advances in Cereal Science and Technology*; Pomeranz, Y., Ed.; American Association of Cereal Chemists: St. Paul, Minnesota, 1990, Vol. 10, pp 143–307.
- Bressani, R.; Turcios, J. C.; de Ruiz, A. S. Nixtamalization effects on the contents of phytic acid, calcium, iron and zinc in the whole grain, endosperm and germ of maize. *Food Sci. Technol. Int.* **2002**, *8*, 81–86.

- (5) Bressani, R. Chemistry, technology and nutritive value of maize tortillas. *Food Rev. Int.* **1990**, *6*, 225–264.
- (6) Bressani, R.; Marengo, E. The enrichment of lime-treated corn flour with proteins, lysine and tryptophan and vitamins. *J. Agric. Food Chem.* **1963**, *6*, 517–522.
- (7) Reinhold, J. G.; García-López, J. S.; Garzon, P. Binding of iron by fiber of wheat and maize. *Am. J. Clin. Nutr.* **1981**, *34*, 1389–1392.
- (8) Reinhold, J. G.; García-López, J. S. Fiber of the maize tortilla. *Am. J. Clin. Nutr.* **1979**, *32*, 1326–1329.
- (9) Martínez-Torres, C.; Taylor, P.; Leets, I.; Tropper, E.; Ramírez, J.; Layrisse, M. Iron absorption from maize bread. *Food Nutr. Bull.* **1987**, *9*, 62–69.
- (10) Wyatt, Jane C.; Triana-Trejos, A. Soluble and insoluble Fe, Zn, Ca, and phytates in foods commonly consumed in northern Mexico. *J. Agric. Food Chem.* **1994**, *42*, 2204–2209.
- (11) Gomez-Aldapa, C. A.; Martínez-Rustos, F.; Figueroa-Cardenas, J. D.; Orodrica Falomir, C. A.; González-Henández, J. Cambios en algunos componentes químicos y nutricionales durante la preparación de tortillas de maíz elaboradas con harinas instantaneas obtenida por extrusion continua. *Arch. Latinoam. Nutr.* **1996**, *46*, 315–319.
- (12) Urizar, A. L.; Bressani, R. Efecto de la nixtamalización del maíz sobre el contenido de ácido fítico, calcio y hierro total y disponible. *Arch. Latinoam. Nutr.* **1997**, *47*, 217–223.
- (13) Khan, N.; Zamora, R.; Elaki, M. Effect of heat treatment on the phytic acid content of maize products. *J. Sci. Food Agric.* **1991**, *54*, 153–156.
- (14) AOAC. *Official Methods of Analysis*, 15th ed.; Association of Official Analytical Chemists: Washington, DC, 1990.
- (15) Haug, W.; Lantzch, H. J. Sensitive method for the rapid determination of phytate in cereals and cereal products. *J. Sci. Food Agric.* **1983**, *54*, 1423–1426.
- (16) Trejo-Gonzalez, A.; Feria-Morales, A.; Wild-Altamirano, C. The role of lime in the alkaline treatment of corn for tortilla preparation. *Adv. Chem. Ser.* **1982**, *No. 19.8*, 245–263.
- (17) Zazueta, C.; Ramos, G.; Fernández-Muñoz, J. L.; Rodríguez, M. E.; Acevedo-Hernández, G.; Pless, R. C. A radioisotope study of the entry of calcium into the maize kernel during nixtamalization. *Cereal Chem.* **2002**, *79*, 500–503.
- (18) O'Dell, B. L.; de Boland, A. R.; Koirtiyohann, S. R. Distribution of phytate and nutritionally important elements among the morphological components of cereal grains. *J. Agric. Food Chem.* **1972**, *20*, 718–721.
- (19) De Boland, A. R.; Garner, G. B.; O'Dell, B. L. Identification and properties of “phytates in cereal grains and oil seed products”. *J. Agric. Food Chem.* **1975**, *23*, 1186–1190.
- (20) Mendoza, C.; Viteri, F. E.; Lonnerdel, B.; Young, K. A.; Raboy, V.; Brown, K. H. Effect of genetically modified low-phytic acid maize on absorption of iron from tortillas. *Am. J. Clin. Nutr.* **1998**, *68*, 1123–1127.
- (21) Hallberg, L.; Brune, M.; Erlandsson, M.; Sandberg, A. S.; Rossander-Hulten, L. Calcium: effect of different amounts on non heme and heme-iron absorption in humans. *Am. J. Clin. Nutr.* **1991**, *53*, 112–119.
- (22) Hallberg, L.; Rossander, L.; Hulten, B. M.; Gleerup, A. Inhibition of haem iron absorption in men by calcium. *Br. J. Nutr.* **1992**, *69*, 533–540.

Received for review September 4, 2003. Revised manuscript received November 15, 2003. Accepted November 17, 2003. This research was conducted with partial financial support from the Consejo Nacional de Ciencia y Tecnología (CONCYT) de Guatemala (Project 62-98).

JF030636K